American Journal of Medical Genetics 61:182–187 (1996)

Cytogenetic and Molecular Analysis of inv dup(15) Chromosomes Observed in Two Patients With Autistic Disorder and Mental Retardation

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A variety of distinct phenotypes has been associated with supernumerary inv dup(15) chromosomes. Although different cytogenetic rearrangements have been associated with distinguishable clinical syndromes, precise genotype-phenotype correlations have not been determined. However, the availability of chromosome 15 DNA markers provides a means to characterize inv dup(15) chromosomes in detail to facilitate the determination of specific genotype-phenotype associations. We describe 2 patients with an autistic disorder, mental retardation, developmental delay, seizures, and supernumerary inv dup(15) chromosomes. Conventional and molecular cytogenetic studies confirmed the chromosomal origin of the supernumerary chromosomes and showed that the duplicated region extended to at least band 15q13. An analysis of chromosome 15 microsatellite CA polymorphisms suggested a maternal origin of the inv dup(15) chromosomes and biparental inheritance of the two intact chromosome 15 homologs. The results of this study add to the existing literature which suggests that the clinical phenotype of patients with a supernumerary inv dup(15) chromosome is determined not only by the extent of the duplicated region, but by the dosage of genes located within band 15q13 and the origin of the normal chromosomes 15. © 1996 Wiley-Liss, Inc.

KEY WORDS: inv dup(15) chromosomes, chromosome 15 biparental inheritance

INTRODUCTION

Supernumerary inv dup(15) chromosomes are a common cytogenetic finding. A review indicates that one-half of all supernumerary marker chromosomes are derived from chromosome 15 and approximately 80% of these represent an inv dup(15) chromosome [Mattei et al., 1984; Buckton et al., 1985]. However, since these markers demonstrate significant clinical and cytogenetic heterogeneity, these chromosomes represent a dilemma in clinical cytogenetics. Although inv dup(15) chromosomes have been observed in the karyotypes of phenotypically normal individuals [Stetten et al., 1981; reviewed in Webb, 1994], they are more commonly associated with moderate to severe mental retardation, developmental delay, behavior problems, and seizures, with or without structural malformations [Wisniewski et al., 1979; Maraschio et al., 1981; Buckton et al., 1985]. The presence of inv dup(15) chromosomes has been also associated with the Prader-Willi syndrome (PWS) [reviewed in Mattei et al., 1984; Robinson et al., 1993a] and Angelman syndrome (AS) [Robinson et al., 1993a].

The mechanism(s) whereby multiple copies of loci on proximal 15q result in a variety of different clinical phenotypes remains an enigma. Efforts to derive genotype-phenotype correlations in patients with an extra chromosome, and to characterize the size and composition of inv dup(15) chromosomes, have previously relied almost exclusively on cytogenetic analysis. Past studies suggested that the observed clinical variability may be the consequence of the amount and parental origin of the DNA retained in the inv dup(15) marker chromosome. This hypothesis was supported by previous clinical studies that demonstrated that patients with the largest markers, inv dup(15)(q12 or q13), usually have varying degrees of mental deficiency and relatively minor somatic anomalies [Wisniewski et al., 1979; Maraschio et al., 1981; Mattei et al., 1984]. In contrast, patients with smaller markers, inv dup(15)(q11), were found in normal individuals [Stetten et al., 1981; Maraschio et al., 1988; Leana-Cox et al., 1994] and in

Received for publication May 16, 1995; revision received August 1, 1995.

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patients with PWS [Wisniewski et al., 1980; Maraschio et al., 1981; Mattei et al., 1984; Buckton et al., 1985]. However, based on conventional cytogenetic analysis alone, it has been difficult to determine the exact breakpoints involved.

The availability of molecular markers derived from chromosome 15 provides a means to define further correlations between clinical phenotype and the inv dup(15) chromosome genotype. Recent molecular analyses have suggested that the number of additional copies of the PWS critical region mapped within bands 15q11–q13, rather than the extent of the duplication, may be a major factor determining clinical severity of the inv dup(15) phenotype [Robinson et al., 1993a,b; Leana-Cox et al., 1994; Cheng et al., 1994].

In this report we describe the cytogenetic and molecular characterization of the supernumerary inv dup(15) chromosomes found in two patients presenting with non-specific mental and developmental retardation, autistic behavioral abnormalities, seizures, and minor phenotypic abnormalities. GTG-banding and distamycin A/DAPI staining confirmed the chromosomal origin of the supernumerary chromosomes and fluorescence in situ hybridization (FISH) analysis determined the DNA content of each. By performing PCR analysis of patient DNA, polymorphic CA dinucleotide repeats located along chromosome 15 showed the parental origin of the inv dup(15), as well as the origin of both normal chromosomes 15.

MATERIALS AND METHODS

Patients

Patient JB, a male, was born at term with a birth weight of 3.94 kg and a length of 50 cm. Reduced fetal activity was recorded during the pregnancy. The patient was breast-fed for 6 months without difficulty and with normal weight gain. Hypotonia and delayed motor and language development were noted during infancy. The patient sat independently at 12 months, vocalized at 18 months, and walked at 24 months. During childhood he displayed abnormal behavior including self-stimulation, echolalia, and repetitive obsessive activities. A unilateral strabismus was corrected surgically. Autism was diagnosed at 6 years. Psychological evaluations estimated his IQ to be 50. Generalized tonic-clonic seizures were recognized at 10 years. An EEG was nondiagnostic; normal diagnostic studies included a head MRI scan, an electromyogram, and nerve conduction velocities.

Examination at 13 years showed mild motor and moderate mental retardation with slight truncal hypotonia. The patient was obsessive and intolerant of altered patterns of activity. Weight, height, head circumference, and hand length were between the 75th and 95th centiles. Mild truncal obesity was observed as was dolichocephaly, bitemporal narrowing, and ridging of the metopic suture. Normal male genitalia were present; the patient was Tanner stage 3.

Patient MB, a female, was ascertained by pediatric psychiatry for autistic behavior at age 6 years. Specific details regarding her prenatal and early medical history were unavailable. She was born to a 28- and 29-year-old mother and father, respectively, following a term pregnancy complicated by first trimester bleeding. Two older sibs were healthy. Hypotonia and developmental delays were noted during infancy. Seizures were diagnosed at 5 months. Bilateral strabismus was surgically corrected. Psychological testing estimated her IQ to be 40. The patient displayed abnormal behavior, including self-stimulation, echolalia, and contact avoidance. A head CT scan and an EEG were normal. Based on the behavioral symptoms, a diagnosis of autistic disorder was made. The clinical details were described elsewhere [Ghaziuddin et al., 1993].

Examination at 7 years showed moderate motor and mental retardation with mild truncal hypotonia. The patient was microcephalic (OFC = 49.5 cm); weight, height, and hand length were between the 3rd and 25th centiles. Several unusual physical findings included facial asymmetry, a flat occiput, bilateral epicanthal folds, simplified ears with absent antihelixes, and a short anteverted nose. Fifth digit clinodactyly was present.

Cytogenetics

Peripheral blood lymphocytes from the patients and their parent(s) were cultured to obtain early-metaphase chromosomes by standard cytogenetic procedures. Cells were synchronized using an FUdR (10^{-5} M) block for 17 hr and a thymidine (10^{-7} M) release for 4 hr, followed by exposure to colcemid (0.07 μg/ml) for 20 min. Metaphase chromosomes were examined by Trypsin G-banding [Seabright, 1971] and distamycin A/DAPI staining [Schweizer et al., 1978].

FISH (Fluorescence In Situ Hybridization)

Metaphase chromosomes from EBV-transformed lymphoblastoid cell lines derived from each patient were used for FISH analysis. The probes used for FISH included a yeast artificial chromosome (YAC) clone (B94H7, St Louis YAC library) containing the DNA marker D15S24 (CMW-1) mapped distal to the PWS critical region in band 15q13 [Malcolm and Donlon, 1994], probe D15Z1 representing chromosome 15 p-arm classical satellite DNA (Oncor, Gaithersburg, MD) and cosmids containing the Prader-Willi/Angelman syndrome region A (D15S11), B (GABRB3), C (SNRPN), and region D (D15S10) (Oncor, Gaithersburg, MD). Cosmids A, B, C, and D all mapped within bands 15q11–q13 [Kuwano et al., 1992] and each probe included a chromosome 15 control cosmid (PML) mapped to 15q22. Hybridization conditions for D15Z1 and for each cosmid was performed as described by the manufacturer. Hybridization of the YAC clone (CMW-1) was carried out with 150–200 ng biotin-labeled DNA precipitated with 15–20 μg human Cot-1 DNA, and resuspended in a 10 μl volume containing 50% formamide, 10% dextran sulfate 2 × SSC (pH 7.0). Following probe denaturation and preannealing for 3 hr at 37°C, probe hybridization and signal detection were achieved as described [Flejter et al., 1995]. Slides were viewed using a Zeiss Axioskop microscope. Photographs were taken with Kodak Ektachrome 400 film. A total of 20 metaphase cells were scored for each probe. Probe hybridization to the two normal chromosome 15 homologs served as a positive control.

Dinucleotide Repeat Polymorphism

Genomic DNA isolated from EBV-transformed lymphoblastoid cell lines was used for PCR analysis of microsatellite repeat polymorphisms with primers generated to PCR amplify DNA markers 15S131 (AFM262xb1) [Malcolm and Donlon, 1994] and GABRB3 ($GABA_A$ receptor β_3) [Mutirangura et al., 1992]. Primers to the CA strand of D15S131 and to the GT strand of GABRB3 were end labeled with γ ^{32}P-ATP for 10 min at 37°C in a volume of 10 μl containing 100 ng primer, 10 mM $MgCl_2$, 5 mM DTT, 70 mM Tris-HCl, and 10 units of T4 polynucleotide kinase. PCR reactions were performed in a total volume of 10 μl containing 10 ng of genomic DNA, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 60 nM each primer, and 0.1 U Taq DNA polymerase. Amplifications were performed on the Perkin-Elmer/Cetus 9600 Thermocycler with initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C (30 sec), annealing at 62°C (30 sec), extension at 72°C (30 sec), and a final extension of 72°C for 10 min. Reaction products were separated on 6% denaturing polyacrylamide gels and then detected by autoradiography using standard methods.

RESULTS

Cytogenetics

Karyotype analysis by high resolution G-banding showed that both patients had 2 structurally normal chromosome 15 homologs and a relatively large supernumerary bisatellited marker chromosome in each of 20 cells examined. Variations in the length of the stalk and satellite regions of the inv dup(15) chromosomes were noted, but no distinct polymorphisms were observed. The distamycin A/DAPI staining technique demonstrated the presence of 2 fluorescent bands on either end of the marker chromosomes confirming that they were derived from chromosome 15 (data not shown). The G-band pattern of the inv dup(15) chromosomes suggested that each contained two copies of band 15q12 and a relatively large light banded region that probably represented band 15q13 (Fig. 1). Thus, the karyotypes were designated as 47,X(X or Y),+inv

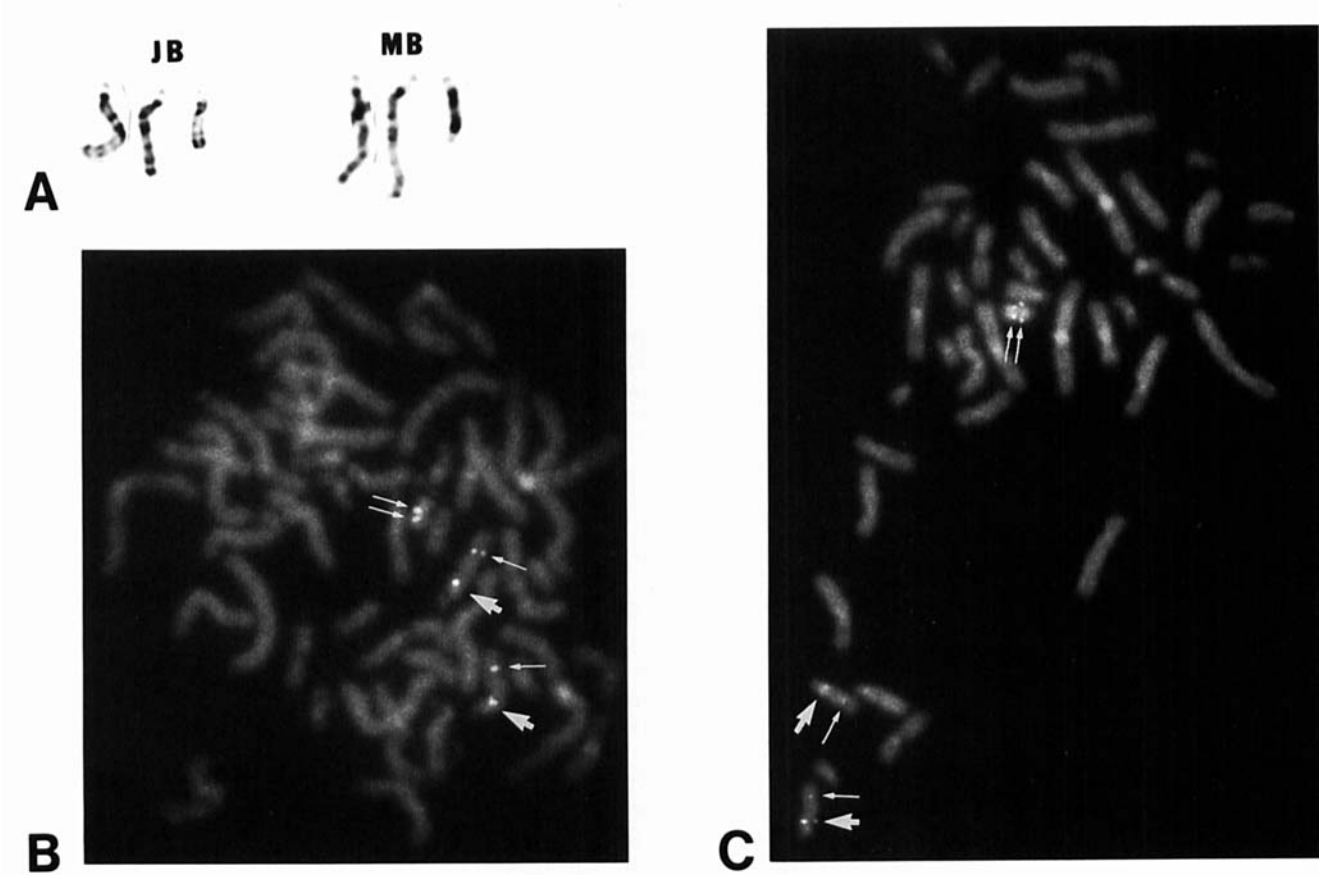

Fig. 1. **A:** Partial GTG-banded karyotypes of two patients (JB and MB) with autistic disorder, mental retardation, and additional supernumerary inv dup(15) chromosomes. In each karyotype the normal chromosome 15 homologs are on the left and center, and the inv dup(15) is on the right. The breakpoints of each inv dup(15) appear to lie within band 15q13. **B,C:** Hybridization of DNA marker GABRB3 (PWS region B) to metaphase chromosomes from patient MB (B) and JB (C) showed two hybridization sites on the inv dup(15) marker chromosomes in each patient (double arrows) and a single hybridization site on the proximal long arm of both normal chromosome 15 homologs in the region 15q11-q13 (large arrows). The hybridization signals observed on the distal long arm of chromosome 15 (small arrows) represent a control probe (PML) previously mapped to 15q22 (Oncor, Gaithersburg, MD).

dup(15)(pter-q13::q13-pter). Parental karyotypes were normal in both families, suggesting that the patient rearrangements were de novo.

FISH

FISH was used to more definitively determine the size of chromosome 15 segments contained in the inv dup(15) chromosomes using YAC and cosmid clones known to contain loci D15Z1, D15S11, SNRPN, D15S10, GABRB3 and D15S24 (Table I, Fig. 1). The results of the FISH analysis are summarized in Table I. An analysis of metaphase chromosomes from MB showed the presence of 2 copies of DNA markers D15Z1, D15S11 and SNRPN on the inv dup(15), and on both normal chromosome 15 homologs, in each of 20 metaphase cells examined. Not all cells examined with probes for DNA markers D15S10, GABRB3, and D15S24 showed 2 fluorescent signals on the inv dup(15). These results may reflect the efficiency of hybridization or variations in chromosome length and, hence, the ability to resolve two fluorescent signals located in close proximity to one another on the inv dup(15) chromosome. In either case, the identification of some cells with two signals on the inv dup(15) chromosome using the most distal probe, D15S24, suggested that both arms of the marker were relatively symmetrical and that the duplicated region extended within or distal to band 15q13.

An analysis of metaphase chromosomes from JB with the same 6 probes demonstrated that 2 copies of markers D15Z1 and D15S11 were present on the inv dup(15) chromosomes in all cells examined. As with patient MB, hybridizations with SNRPN, D15S10, and GABRB3 showed 2 fluorescent signals on the inv dup(15) chromosome in most cells examined. In contrast, only one copy of D15S24 was resolved on the inv dup(15) in eight of 20 cells scored and in the remaining cells no signal was observed. These results may suggest that the inv dup(15) in this patient was asymmetrical, containing only a single copy of this region yet containing two copies of the PWS critical region. Alternatively, since metaphase preparations were relatively condensed, only a single fluorescent signal may have been discerned due to the limits of chromosome resolution.

Microsatellite Repeats

For each patient, the parental origin of the 2 normal chromosome 15 homologs and of the inv dup(15) chromosome was determined by PCR analysis of CA polymorphic repeats (Fig. 2). Probe D15S131, localized outside the inv dup(15) region within band 15q23-q24, detected a 5-allele polymorphism that was informative in both families. In family I, both parents were heterozygous with the father having alleles A and E and the mother having alleles B and D. JB was heterozygous for alleles B and E, demonstrating biparental inheritance of the two normal chromosome 15 homologs. In family II, the patient's father was heterozygous having both a C and an E allele; the patients genotype was B/E. These findings suggested that the patient's B allele was inherited from her deceased mother. However, since maternal DNA was not available, we cannot exclude the possibility of maternal heterodisomy.

A second $(CA)_n$ polymorphism closely linked to the GABRB3 locus, and located within band 15q11-q13, detected a 6-allele polymorphism that identified the likely paternal origin of the inv dup(15) chromosome. In family I, the proband's father had alleles E and F while the mother had alleles A and D. The father transmitted his F allele from a normal chromosome 15 to his affected son (JB) and the mother transmitted both her A and D alleles; one present on a normal chromosome 15 and a second on the inv dup(15). Although the FISH data indicated that 2 copies of the GABRB3 locus were present on the inv dup(15), no obvious dosage difference in maternal alleles was discerned. However, the presence of two different maternal alleles, in addition to the results observed with D15S131 supported a maternal origin of the inv dup(15) in this patient; therefore the genotype for this patient was designated as A/D/F/?. Similar results were observed in family II. MB's father, heterozygous for alleles C and F, transmitted his F allele to his affected daughter who also inherited both a B and a D allele from her obligatory heterozygous mother; these results, in conjunction with those obtained with marker D15S131 would be consistent with a maternal origin of the inv dup(15) chromosome in this patient.

DISCUSSION

In this study we have described the cytogenetic and molecular analyses of relatively large bisatellited supernumerary chromosomes identified in 2 patients with autistic disorder, mental retardation, developmental delay, behavior problems, seizures, and minor physical anomalies. Conventional and molecular cytogenetic studies showed that the markers were derived from chromosome 15. In one patient the inv dup(15)

TABLE I. FISH Analysis of inv dup(15)

Patient	No. of signals on inv dup(15)[b]	No. of positive cells observed with each probe[a]					
		D15Z1	D15S11	SNRPN	D15S10	GABRB3	D15S24
MB	0	0	0	0	0	0	0
	1	0	0	0	3	10	17
	2	20	20	20	17	10	3
JB	0	0	0	0	0	0	12
	1	0	0	4	2	7	8
	2	20	20	16	18	13	0

[a]Twenty metaphase cells were scored per hybridization.
[b]Hybridization signals were present on both normal chromosome 15 homologs in all cells examined.

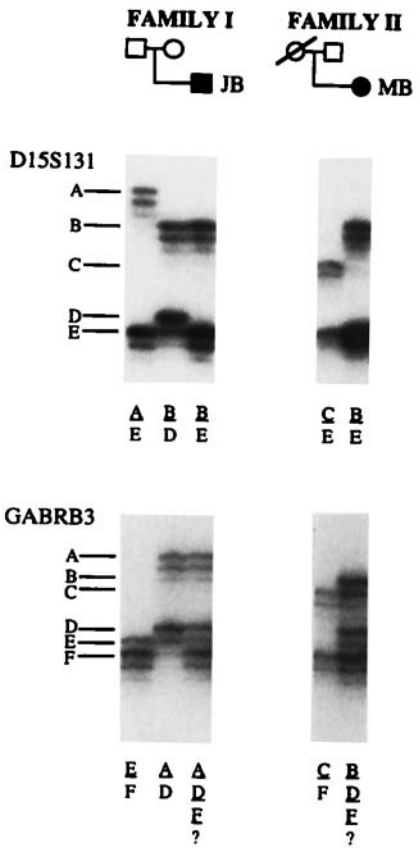

Fig. 2. PCR analysis of patients (JB and MB) and their parent(s) with polymorphic CA repeats for D15S131 located within bands 15q23-q24, and with GABRB3 located within bands 15q11-q13. Alleles produced by the PCR typing are labeled to the left of each autoradiograph. The genotype of each individual is listed below their respective lanes.

contained two copies of DNA markers spanning from the centromere to marker CMW-1, indicating that the breakpoints extended within or distal to band 15q13. The inv dup(15) chromosome in the second patient appeared to contain two copies of markers ranging from the centromere to GABRB3 but only one additional copy of the most distal marker, CMW-1. These results may indicate that the breakpoint of the inv dup(15) lies within or slightly distal (<1 Mb) to CMW-1 therefore, only a single hybridization signal was detected. In one case, microsatellite repeat polymorphisms demonstrated biparental inheritance of the two normal chromosome 15 homologs and indicated a maternal origin of the inv dup(15) chromosome. Similar results were observed in the second case although maternal DNA was not available for study and we cannot conclusively rule out maternal heterodisomy of the normal chromosome 15's with a paternally derived inv dup(15). However, no paternally derived inv dup(15) chromosomes large enough to include the PWS region have been reported [Robinson et al., 1993b].

An association between the phenotype and genotype in patients with an inv dup(15) has previously been proposed based on an analysis of 46 patients: 38 by FISH [Leana-Cox et al., 1994; Cheng et al., 1994] and eight by molecular RFLP analysis [Nicholls et al., 1989; Shibuya et al., 1991; Robinson et al., 1993a,b). In a relatively large study, Leana-Cox et al. [1994] used FISH to demonstrate that patients with an inv dup(15) chromosome lacking markers mapped to band 15q12 were normal, whereas those patients with inv dup(15) chromosomes with one or more additional copies of markers in the region had an abnormal phenotype. Robinson et al. [1993b] suggested further that patients trisomic for DNA markers located in or more distal to band 15q12 demonstrated only moderate mental retardation and mild physical handicaps. In contrast, Robinson et al. [1993b] showed that patients tetrasomic for markers mapped more distal to band 15q12 showed moderate to severe mental retardation, autistic behavior problems, epilepsy, and moderate motor retardation. The present study complements and extends the past analyses of inv dup(15) marker chromosomes using a number of cytogenetic and molecular techniques. The results are consistent with those described by Robinson et al. [1993b] showing that the inv dup(15) chromosomes in our two patients are tetrasomic for markers located within the PWS critical region and that each patient exhibits significant mental retardation, autism, and epilepsy with mild somatic abnormalities. Together, with previous reports, these studies provide additional observations to begin to derive the correlations between clinical phenotype and the inv dup(15) marker chromosome genotype.

ACKNOWLEDGMENTS

We thank Steve Maynard and Diane Miller for their technical assistance, and our patients and parents for their cooperation. Probes used for the FISH analyses were generously provided by the University of Utah Cytogenetics Laboratory. This work was supported by the State of Michigan Department of Public Health grant 38-6006309 and the NIH Center for Genomic Technology and Genetic Disease, Somatic Cell Genetics Core grant P50-HG00209 to J.L.G.

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